PATENT SPECIFICATION 812,402

The inventor of this invention in the sense of being the actual deviser thereof within the meaning of Section 16 of the Patents Act, 1949 is KARL JOHAN STAMPE VILLADSEN, a Danish Subject, of No. 5 Otto Benzonsvej, Arhus, Denmark.

Date of filing Complete Specification : May 23, 1955.

Application Date : May 25, 1954. No. 15419/54.

Complete Specification Published : April 22, 1959.

Index at Acceptance :—Class 14(2), E4.

International Classification :—C12h.

COMPLETE SPECIFICATION.

Improved Process for Clarifying Fermented Liquor.

We, AKTIESELSKABET GRINDSTEDVAERKET, a Company organised under the laws of Denmark, of 53 Jens Baggesensvej, Aarhus, Denmark, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:

This invention relates to the clarification of grape juices, wine must under fermentation with or without the pulp and skin of grapes left therein, or fermented must or wine, and particularly to the reduction of metal compounds such as compounds of iron, copper magnesium or calcium contained in such juice or must or wine in order to improve the keeping qualities.

Clarification processes consisting in the treatment of natural juice with enzymes such as amylase or proteolytic or pectolytic enzymes or mixtures thereof which have found extensive use in the treatment of juices of apple, gooseberry, strawberry and other fruits have been found of very little use in the treatment of the juice of grape in the manufacture of wine.

The clarification of the juice of wine is thus normally a natural process the successful progress of which is, however of great importance for the stabilisation of the must and the wine, since a prolonged sedimentation may be the cause of malady or sickness of the wine or other deteriorative changes. The clarification is often brought about by filtering or centrifugal action which may assist or promote natural sedimentation in some cases, but in other cases fail to be of any assistance. The reason for this is that the must or wine contains colloidal substances which prevent the sedimentation and form eventually a substratum for the development of undesirable microorganisms. They also tend to clog the filters or prevent centrifugal action or decrease the effect of the filtering of centrifugal processes. The most important of these substances are those related to pectin.

Since the nature and properties of these substances vary during the treatment of the grape juice, must and wine the amount of substances that produce unclearness held in suspension or pseudo-solution by them may vary and the formation of sediment may continue after clarification, filtration or centrifugation if they are not also removed or decomposed. It has been found that during the natural clarification of grape juices, musts or wine a decomposition of the substances in question, particularly the pectic substances, takes place so that a clear wine will be almost or completely deprived of them. Decomposition or removal of these substances by artificial means is therefore only a promotion of the natural clarification process.

It has been found, however, that not all enzymes capable of producing clarification of fruit juices are equally well suited for the clarification process to be carried out in the manufacture of wine and the invention relates to enzyme complexes, mainly of pectolytic nature as hereinafter defined, producing a particularly good clarification and stabilisation of wine must and wines, and at the same time reducing considerably the contents of metal compounds thereof, thereby improving the keeping qualities of the wine.

The pectolytic enzyme complexes to be used according to the invention are characterised by being capable of throwing down chlorophyll from aqueous solutions of chlorophyll at a *p*H of 3.0 to 4.5, a temperature of 25 to 30° C. within a time of up to 40 hours.

Preferably also amylase and/or proteinase are added with the pectolytic enzyme com-

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plex to the wine, must or wine as these substances are of value in hydrolysing undesirable starch-like proteinaceous substances which may be present.

The enzyme complex in question may be produced by the action of suitable strains of Aspergillus niger or Penicillia upon the usual substrates used for growing these organisms for producing pectolytic substances, but a strain is selected the enzymes of which have the above named properties. The enzyme complex may be used in the form of the substrate upon which the organism has been grown which substrate may contain the living organism or not or in the shape of an aqueous extract of the substrate evaporated upon a water-soluble extender such as lactose, glucose or other sugars or in other forms.

According to the present invention therefore there is provided a method of clarifying grape juices, wines or must under fermentation with or without the pulp or skin of grapes left therein or fermented must which comprises adding to the juice, wine must, fermented must or wine a pectolytic enzyme complex characterised by being capable of throwing down chlorophyll from aqueous solutions of chlorophyll at a *p*H of 3.0 to 4.5, a temperature of 25° to 30° C. and within a time of up to 40 hours, leaving the juice, must or wine until the main fermentation has been finished and then decanting and centrifuging or in the case of fermented must or wine leaving for two to four weeks and then decanting, filtering or centrifuging.

Also according to the invention amylase and/or proteinase is preferably added to the juice, wine must, fermented must or wine together with the pectolytic enzyme complex.

The enzyme used for the test must also be dissolved in a buffered solution at the same *p*H. The test can be carried out in the following manner.

0.5 gram of chlorophyll of the kind usually designated as water-soluble is dissolved as far as possible in 400 ml. of a solution of a citric acid buffer at *p*H 3.2 and at a temperature of 25° C. Only a part of the chlorophyll dissolves and accordingly the solution must be filtered before use.

A solution is produced from the enzyme complex to be tested. For this purpose 1 gram of the enzyme complex is dissolved in 100 mls. of a citric acid buffer solution at *p*H 3.2. If the enzyme complex to be tested contains an insoluble substrate it must be subjected to extraction with the buffer solution by being mixed therewith and left to stand for 1½ hours at room temperature with frequent stirring. The extract is filtered and the filtrate is used for the test.

In carrying out the test 25 mls, of the chlorophyll solution produced as described above is mixed with 1 ml. of a solution of the enzyme complex produced as described above. The solution is left to stand at 25° C. In some cases a separation of chlorophyll will occur after about 20 hours. If no such separation appears 2 millilitres of the solution of the enzyme complex to be tested are further added and the mixture is again left to stand at 25° C. If during a further lapse of not more than 20 hours a separation of chlorophyll or other metal-containing precipitate appears the enzyme complex can be used in accordance with the present invention. If no precipitate has appeared either during the first or the second lapse of 20 hours each the enzyme cannot be so used.

If the test is carried out as described with amylase alone or with any other commercially available pectolytic enzyme alone no precipitation will be obtained.

This test thus clearly differentiates the pectolytic enzyme complexes to be used according to the invention from other pectolytic enzyme complexes.

In using the particular enzyme complex in the manufacture of wine the enzyme complex may be added to the crushed grapes or the must containing the skin or pulp of the grapes or not and left therewith until the main fermentation has been finished, after which decanting, filtering or centrifuging takes place. It may also be added to wine or fermented must which is then left with it for 2 to 4 weeks at normal storing or ageing conditions and subsequently decanted, filtered or centrifuged. Other manners of utilizing the new enzyme will suggest themselves in practice.

The enzyme preparations may be used in very pure and concentrated form or precipitated on some suitable vehicle, for instance bran or husks of various cereals, such as wheat, rye or rice or on various other insoluble substrates, for instance diatomaceous earth provided it is sufficiently pure and free of iron or on a soluble substrate, for instance glucose or lactose.

In most cases there is advantageously added 20—50 grams of the enzyme complex to 100 kilograms of the grape juice, must or wine, but in many cases a further advantage can be obtained by using 100 g. or more per 100 kilograms.

As a result of the action of the pectolytic enzyme complex the must or wine will have an improved clearness and stability and the contents of iron and perhaps also of copper and in some cases of calcium will be considerably reduced. Thus in the case of wine produced from blue grapes the decrease in contents of iron salts amounted to 30% or more. In some cases where the enzyme complex is added to the must containing the skin and pulp of the grape it has been found that a considerably greater yield of must has

been obtained than without the use of enzyme complex.

The increased clearness or the promotion of clarification of the must or wine, the increased yield and the decrease in the contents of metal compounds represent important advantages connected to the use of the new enzyme complex in the manufacture of wine.

The following Examples will serve to illustrate the new effect that can be obtained by using the invention in various treatments, the manner of treatment, the conditions under which the same is carried out, the kind of raw material and other facts connected with the treatment being mentioned by way of example only and the beneficial effect of the treatment being not limited to the facts mentioned.

EXAMPLE 1.

A must of grapes characterised by the following analytical data is divided into six equal parts, two of which are used for each of the Examples 1, 2 and 3:—

Sugar	19.1	g. per 100 millilitres
Acids, total	7.6	g. per litre (calc. as tartaric acid)
Acids, volatile	0.12	g. per litre (calc. as acetic acid)
Nitrogen, total	0.257	g. per litre
Iron, total	24.00	milligrams per litre
pH	3.35	

To both portions of the must used for this example are added 10 grams of potassium metal bisulphite to each 100 litres. To one of the portions a preparation of pectolytic enzyme complexes according to the invention precipitated on a soluble substrate is added in the proportion of 100 grams to each 100 litres. After fermentation for 5 weeks the iron content has decreased to 14 milligrams per litre. The other portion having been subjected to fermentation for the same period of time under the same conditions without the addition of the enzyme preparation contains 21.25 milligrams iron per litre. After a further fermentation period of 5 weeks the iron content in the former portion has decreased to 11.5 milligrams per litre, whereas in the latter portion it has only decreased to 21 milligrams per litre.

The alcohol content in the former portion is 9.8% whereas in the latter portion it is only 9.2%.

EXAMPLE 2.

To one portion of the same must as in Example 1 another enzyme preparation according to the invention containing in addition to pectolytic enzymes also amylase and proteinase is added in the proportion of 100 grams to each 100 litres. Both portions are subjected to fermentation.

After 5 weeks the iron content in the must containing the enzymatic preparation according to the invention has decreased to 13.25 milligrams per litre, whereas in the must that has had no addition of the enzymatic preparation the iron content is 21.25 milligrams per litre.

After a further 5 weeks of fermentation the iron contents are 9.00 and 21.00 milligrams per litre, respectively, and the alcohol contents 9.3 and 9.2%, respectively.

EXAMPLE 3.

To a portion of the same must as mentioned in Example 1 an enzymatic preparation containing pectolytic enzymes precipitated on bran of rice and satisfying the conditions according to the invention are added in proportion of 50 grams to each 100 litres of metal and the must is subjected to fermentation. A similar portion of the must is fermented without addition of the enzymatic preparation.

After 5 weeks the contents of iron in the two portions are 19.00 and 21.25 milligrams per litre, respectively. After a further 5 weeks the iron contents are 15.00 and 21.00 milligrams per litre, respectively. The alcohol contents of the wines are 9.9 and 9.2%, respectively.

EXAMPLE 4.

Purple grapes are crushed and the pulp is divided into two portions. To one of the portions 100 grams of an enzyme preparation according to the invention are added to each 100 litres after which it is subjected to fermentation, whereas the other portion is subjected to fermentation without such addition.

After fermentation for 6 weeks the iron contents in the wine are 8 milligrams per litre and 10 milligrams per litre, respectively. The alcohol contents are 12% and 12.3%, respectively.

The quantity of wine obtained in the first pressing is 3% greater in the case of the wine produced from the pulp to which the enzymatic preparation has been added than in the other case.

EXAMPLE 5.

Purple grapes are crushed and the must is recovered by pressing. To the must are added 25 grams of potassium metal bisulphite to each 100 litres after which the must is divided into two portions to one of which 200 grams of an enzymatic preparation according to the present invention precipitated on bran are added to each 100

litres of must. Both portions are subjected to fermentation.

After fermentation the wine contains 2.6 and 4.2 milligrams of iron per litre, respectively, and the alcohol contents are 11.1% and 10.9%, respectively.

WHAT WE CLAIM IS:—

1. A method of clarifying grape juices, wines or must under fermentation or without the pulp and skin of grapes left therein or fermented must which comprises adding to the juice, wine must, fermented must or wine a pectolytic enzyme complex characterised by being capable of throwing down chlorophyll from aqueous solutions of chlorophyll at a *p*H of 3.0 to 4.5, a temperature of 25° to 30° C. and within a time of up to 40 hours, leaving the juice, or wine until the main fermentation has been finished and then decanting and centrifuging or in the case of fermented must or wine leaving for two to four weeks and then decanting, filtering or centrifuging.

2. A method as claimed in Claim 1 in which the proportion of the enzymatic preparation which is added exceeds 20 grams to each 100 kilograms of juice, must or wine.

3. A method as claimed in Claim 1 or 2 in which amylase and/or proteinase is added to the juice, wine must, fermented must or wine together with the pectolytic enzyme complex.

4. The method of clarifying grape juices, wines or must under fermentation substantially as herein described with reference to the foregoing Examples.

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PROVISIONAL SPECIFICATION.

Improved Process for Clarifying Fermented Liquor.

We, AKTIESELSKABET GRINDSTEDVAERKET, a Company organised under the laws of Denmark, of 53 Jens Baggesensvej, Aarhus, Denmark, do hereby declare this invention to be described in the following statement:

This invention relates to the clarification of grape juices, wine must under fermentation with or without the pulp and skin of grapes left therein, or fermented must or wine, and particularly to the reduction of metal compounds such as compounds of iron, copper, magnesium or calcium contained in such juice or must or wine in order to improve the keeping qualities.

Clarification processes consisting in the treatment of natural juice with enzymes such as amylase or proteolytic or peptolytic enzymes or mixtures thereof which have found extensive use in the treatment of juices of apple, gooseberry, strawberry and other fruits has been found of very little use in the treatment of the juice of grape in the manufacture of wine.

The clarification of the juice of wine is thus normally a natural process the successful progress of which is, however, of great importance for the stabilisation of the must and the wine, since a prolonged sedimentation may be the cause of malady or sickness of the wine or other deteriorative changes. The clarification is often brought about by filtering or centrifugal action which may assist or promote natural sedimentation in some cases, but in other cases fail to be of any assistance. The reason for this is that the must or wine contains colloidal substances which prevent the sedimentation and form eventually a substratum for the development of undesirable microorganisms. They also, in consequence of their hydrophilic qualities, tend to clog the filters or prevent centrifugal action or decrease the effect of the filtering or centrifugal processes. The most important of these substances are those related to pectin.

Since the nature and properties of these substances vary during the treatment of the grape juice, must and wine the amount of substance that produce unclearness held in suspension or pseudo-solution by them may vary and the formation of sediment may continue after clarification, filtration or centrifugation if they are not also removed or decomposed. It has been found that during the natural clarification of grape juices, musts or wine a decomposition of the substances in question, particularly the pectic substances, takes place so that a clear wine will be almost or completely deprived of them. Decomposition or removal of these substances by artificial means is therefore only a promotion of the natural clarification process.

It has been found, however, that not all enzymes capable of producing clarification of fruit juices are equally well suited for the clarification process to be carried out in the manufacture of wine and the invention relates to enzymes, mainly of pectolytic nature, producing a particularly good clarification and stabilisation of wine must and wines, and at the same time reducing considerably the contents of metal compounds

thereof, thereby improving in known way the keeping qualities of the wine.

The pectolytic enzymes to be used according to the invention are of a kind acting at comparatively low temperature e.g. 16—18° C. and the *p*H values of 2—5 normally prevailing in wine musts and being characterised moreover by their ability to thrown down in certain test solutions containing organic metal compounds a deposit containing the metal compound in question or the metal-containing part thereof. As test compounds may be used according to the invention haemin or chlorophyll, the former being a water soluble organic iron compound of relatively high molecular weight and the latter being a compound which is closely related to haemin, but which contains magnesium in its molecule in place of iron.

In some cases the enzyme is also capable of sedimenting hematoporphyrin from its aqueous solutions, which substance, although it contains no metal, is closely related to the substance mentioned.

The enzyme in question may be produced by the action of a suitable strain of Aspergillus niger upon the usual substrates used for growing this organism for producing pectolytic substances, but a strain is selected the enzyme of which has the above named properties. The enzyme may be used in the form of the substrate upon which the organism has been grown which substrate may contain the living organism or not or in the shape of an aqueous extract of the substrate evaporated upon a water-soluble extender such as lactose, glucose or other sugars or in other forms. By adding 3 ml. 1% aqueous solution of such enzyme buffered to *p*H 3.2 to a solution or suspension of chlorophyll in a buffer solution of the same *p*H in the amount of 0.03 g. in 25 ml. buffer solution and leaving the solution to stand over night at room temperature all chlorophyll will be precipitated in the form of grains. With the same enzyme solution haemin dissolved in the amount of 0.06 g. in 25 ml. of buffer at *p*H 8.2 will be precipitated. If the same experiment be carried out with amylase or with commercially available pectolytic enzymes differing from the above named one, such as vinibon, pectozyl, panzym or pectinol no precipitation will be obtained.

This test thus clearly differentiates the pectolytic enzymes used according to the invention from other pectolytic enzymes.

In using the particular enzyme in the manufacture of wine the enzyme may be added to the crushed grapes or the must, containing the skin or pulp of the grapes or not, and left therewith until the main fermentation has been finished after which decanting, filtering or centrifuging takes place. It may also be added to wine or fermented must which is then left with it for 2—4 weeks at normal storing or ageing conditions and subsequently decanted, filtered or clarified. Other manners of utilizing the new enzyme will suggest themselves in practice.

In most cases the proportion of the enzyme advantageously exceeds 20 g./per 100 kilograms of the must or wine, 50—60 g./per 100 kilograms being preferable.

As a result of the action of the proteolytic enzyme the must or wine will have an improved clearness and stability and the contents of iron and perhaps also of copper and in some cases of calcium will be considerably reduced. Thus in the case of wine produced from blue grapes the decrease in contents of iron salts amounted to 30%. In some cases where the enzyme is added to the must containing the skin and pulp of the grape it has been found that a considerably greater yield of must has been obtained than without the use of enzyme.

The increased clearness or the promotion of clarification of the must or wine, the increased yield and the decrease in the contents of metal compounds represent important advantages connected to the use of the new enzyme in the manufacture of wine.

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Abingdon: Printed for Her Majesty's Stationery Office, by Burgess & Son (Abingdon), Ltd.—1959.
Published at The Patent Office, 25, Southampton Buildings, London, W.C.2,
from which copies may be obtained.